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Energy Analysis of Zn Polycoordination in a Metalloprotein Environment and of the Role of a Neighboring Aromatic Residue. What Is the Impact of Polarization?

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Abstract: We analyze the intermolecular interaction energies stabilizing the complex of ethanol in the binding site of alcohol dehydrogenase Zn-metalloenzyme (ADH). In this site Zn(II) is ligated by two cysteine and one imidazole residue and by the ethanol substrate. Ethanol is stacked over a phenylalanine residue. The system has been studied by means of SIBFA (Sum of Interactions Between Fragments Ab initio computed) polarizable molecular mechanics (PMM) supplemented by quantum chemical (QC) computations at various levels of theory. The nonadditivities of the QC interaction energies can be traced back by energy-decomposition analyses and are essentially due to polarization, charge-transfer, and electron correlation energies. These contributions can be reproduced by PMM computations. Interestingly, the polarization energy associated with the presence of the benzene ring in the ADH complex is canceled due to many-body/nonadditivity effects. Therefore this ring does not contribute to stabilization prior to including electron correlation/dispersion effects in the QC calculations or in the absence of the PMM dispersion energy contribution. When these effects are taken into account, the stabilization it contributes is in the 3−9 kcal/mol range, reflecting the need for an accurate reproduction of all components of the interaction energy by PMM.

Introduction

Cation-π interactions constitute a widely encountered determinant in molecular recognition. In proteins, they mostly involve the electron-rich Trp, Phe, and Tyr residues and the cationic Arg or Lys residues [reviewed in ref 1]. A novel motif was put forth by Zaric et al.,[2] in which Trp or Phe could indirectly interact with a metal cation, by means of a stacking interaction with a metal ligand. Examples from X-ray crystallography are provided by metalloproteins having Cu(II),[3] Mg(II),[4] Fe(III),[5] or Zn(II)[6] cofactors. This has led us to analyze the energetical factors stabilizing such complexes. We consider here the recognition site of the Zn-metalloprotein alcohol dehydrogenase (ADH), which catalyzes the oxidation of alcohol to aldehyde, and whose crystal structure was published in ref 6. In this site, Zn(II) is bound by four residues, namely two anionic ones, Cys46 and Cys174, and two neutral ones, His67 and the ethanol substrate. Ethanol is stacked over a Phe residue, Phe93.

In this contribution, we propose to address the following points: (1) What are the magnitudes of the intermolecular interaction energies and of their individual contributions

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within the tetracoordinated Zn complex and the amount of additional stabilization contributed by the Phe residue? (2) What is the extent of nonadditivity in the complex and could possibly nonadditivity modulate Phe binding? (3) To what an extent could the magnitudes of the binding energies be affected by the level of the quantum-chemical (QC) computations, and, in the perspective of computations on large proteins, could polarizable molecular mechanics (PMM) such as the SIBFA (Sum of Interactions Between Fragments Ab initio computed) approach satisfactorily match the QC results?

Procedure

QC Computations. We used the Restricted Variational Space Analysis (RVS)[7] to deconvolute Hartree–Fock (HF) intermolecular interaction energies, denoted ΔE(RVS), into four separate contributions: Electrostatic/Coulomb ($E_{\text{Coul.}}$) and exchange-repulsion (E_{exch}) at first-order (denoted as $E_1 = E_{\text{Coul.}} + E_{\text{exch}}$) and polarization ($E_{\text{pol}}$) and charge-transfer ($E_{\text{ct}}$) at second-order (denoted as $E_2 = E_{\text{pol}} + E_{\text{ct}}$). These computations were done using the CEP 4–31G(2d) basis set.[8] Contributions of correlation/dispersion to the total intermolecular interaction energies, ΔE(MP2), were computed by the MP2 procedure[9] using the following approximation:

$$\delta E(\text{MP2}) = \Delta E(\text{MP2}) - \Delta E(\text{HF})$$

$$= \Delta E(\text{correlation}) \sim \Delta E(\text{dispersion}) \qquad (1)$$

This representation should enable evaluation of the efficiency of the SIBFA E_{disp} component since explicit evaluation of dispersion energy by means of Symmetry Adapted Perturbation theory (SAPT[10]) is limited due to the size of the considered systems. We are also aware that electron correlation can affect also the other components of the energy.[10,11]

These computations were done with the GAMESS package.[12] It is to be noted that the RVS procedure as coded in GAMESS removes the Basis Set Superposition Error (BSSE[13]). Thus the reported ΔE(RVS) values are BSSE-corrected at both bi- and multimolecular complexes. The RVS BSSE with the CEP 4–31G(2d) basis set are small, namely, ~5 kcal/mol out of 624. Small relative BSSE values of <1.5% of the interaction energy were previously reported for polycoordinated complexes of a Zn(II) cation.[14] On the other hand, in the computation of δE(MP2), the values of ΔE(MP2)-ΔE(HF) are BSSE-uncorrected. Therefore δE(MP2) embodies those BSSE effects that appear at the MP2 level, and these have larger magnitudes.[15]

Additional DFT computations have been performed using the B3LYP[16] functional. They used the CEP 4–31G(2d) basis set as well as the 6–311G** and LACV3P** basis sets.[17] This latter is equivalent to the 6–311G** basis set on nonmetal atoms. These computations were done with the Gaussian 03 package,[18] except for LACV3P**, where the Jaguar 6.5 software[19] was used. lMP2 computations based on the approach developed by Saebo et al.,[20] as implemented in Jaguar, are also provided at the LACV3P** basis set level and compared to corresponding Jaguar HF values. The BSSE corrections were not done for the 6–311G** and LACV3P** basis sets. Table 2 shows the nonadditivity of CEP 4–31G(2d) BSSE to be small, not exceeding 1.3 kcal/mol. In as much as BSSE has small nonadditivities with other, more extended basis sets as well, this should not affect the analyses of nonadditivity trends.

Table 1. Intermolecular Interaction Energies (kcal/mol) in Complexes *a* and *b*, Which Model the Recognition Site of ADH without and with, Respectively, the Presence of the Benzene Ring[a]

	complex *a* without Phe93		complex *b* with Phe93	
	ab initio	SIBFA	ab initio	SIBFA
$E_{\text{Coul.}}/E_{\text{MTP}}{}^*$	−661.6	−657.9	−664.4	−662.4
$E_{\text{exch.}}/E_{\text{rep*}}$	177.4	168.9	180.2	173.5
E_1	−484.2	−489.0	−484.2	−488.9
E_{pol}(HF)/E_{pol}	−85.5	−94.5	−84.5	−93.2
E_{pol}(RVS)/$E_{\text{pol*}}$	−113.2	−121.7	−111.8	−121.1
E_{ct}(RVS)	−48.5		−48.6	
BSSE	−5.1		−5.6	
$E_{\text{ct*}}/E_{\text{ct}}$	−43.4	−41.3	−43.0	−41.3
E_2	−156.6	−163.0	−154.8	−162.4
$\boldsymbol{\Delta E}$	**−618.2**	**−624.9**	**−617.3**	**−623.4**
δE(MP2)/E_{disp}	−48.4	−65.9	−57.8	−70.5
$\boldsymbol{\Delta E_{\text{tot}}}$	**−666.6**	**−690.8**	**−675.1**	**−693.9**

[a] See text for definition. $E_{\text{pol}}(\text{HF}) = \Delta E - E_1 - E_{\text{ct}}(\text{RVS})$. [This procedure enables us to evaluate a Morokuma-like polarization energy as the KM approach does not converge, as discussed in the text.] $E_{\text{ct*}} = E_{\text{ct}}(\text{RVS}) - \text{BSSE}$. $E_2(\text{HF}) = E_{\text{pol}}(\text{RVS}) + E_{\text{ct*}}$. $E_2(\text{SIBFA}) = E_{\text{pol*}} + E_{\text{ct}}$. $\Delta E(\text{SIBFA}) = E_1 + E_{\text{pol}} + E_{\text{ct}}$.

Polarizable Molecular Mechanics Computations. We have used the SIBFA polarizable force field. Within the SIBFA procedure,[21] the intermolecular interaction energy is computed as a sum of five separate contributions: penetration corrected multipolar electrostatics,[21c] $E_{\text{MTP*}}$; anisotropic short-range repulsion,[21d] E_{rep}; polarization, E_{pol}; charge-transfer, E_{ct}; and dispersion, E_{disp}. Details on the formulation and calibration of these contributions are given in ref 22. The molecular fragments making up the binding site are methanethiolate, imidazole, benzene, and ethanol. They belong to the SIBFA library of fragments. In keeping with our previous studies, the distributed multipoles[23] and polarizabilities[24] are those derived from their HF molecular orbitals computed with the CEP 4–31G(2d) basis set.

Energy minimizations on the internal coordinates used the Merlin package.[25] Because the X-ray structure shows an unrealistically short distance between Zn(II) and the cysteinate (Cy$^-$) 174 S atom, of about 2.0 Å instead of 2.2–2.3, we have optimized the structure in two steps. First, we relaxed the position of Zn(II) inside the cavity and then relaxed simultaneously the Zn(II) cation, the conformation of the ethanol hydroxyl end, and the methanethiolate group representing Cy-174. For this group, the first H atom lying along the C_α-C_β bond and used to anchor the methanethiolate moiety was not relaxed. The energy-minimized structure is shown in Figure 1a.

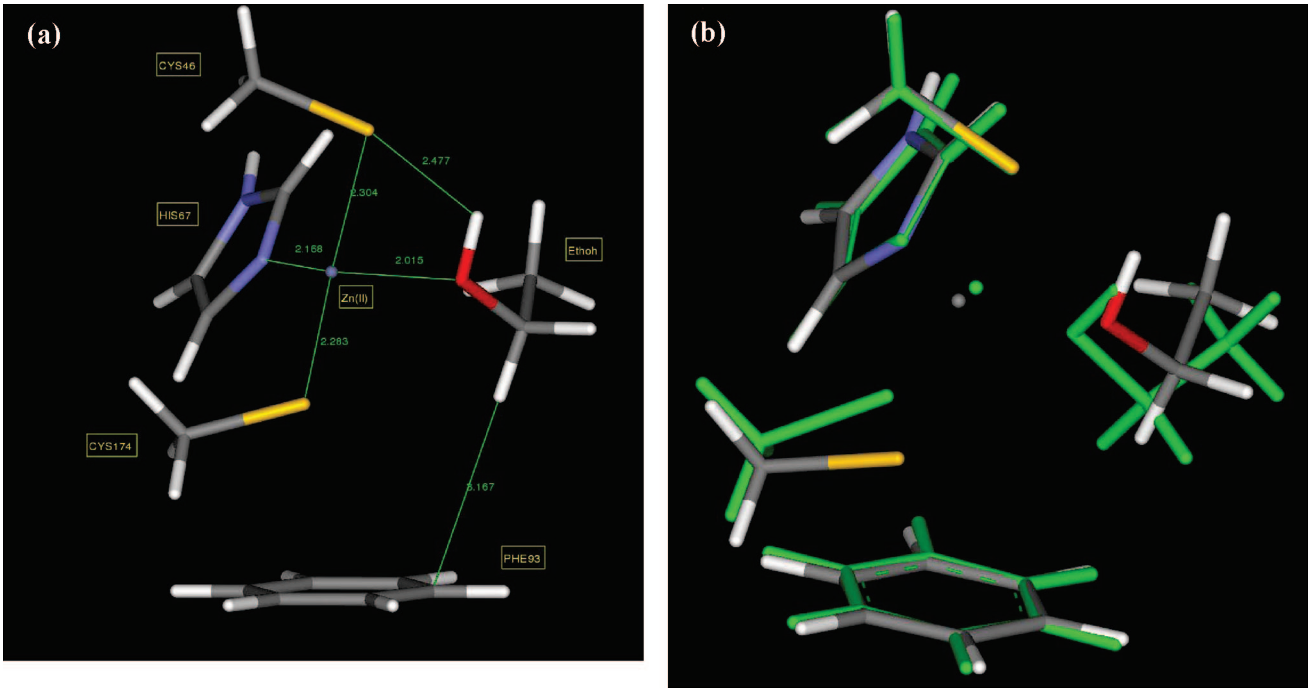

Figure 1. a) Representation of the energy-minimized structure of the recognition site of ADH and b) superimposition of the energy-minimized structures with the PDB structure (in green).

Table 2. Values (kcal/mol) of the RVS/CEP 4-31G(2d) and SIBFA Contributions of the Bimolecular Complexes, of Their Sums and Values of Their Nonadditivities, and of the 6-311G** HF and MP2[a]

	RVS	SIBFA	RVS	SIBFA	RVS	SIBFA	RVS	SIBFA	RVS	SIBFA	RVS			SIBFA	RVS	SIBFA
	$E_{\mathrm{Coul.}}$	$E_{\mathrm{MTP*}}$	$E_{\mathrm{exch.}}$	E_{rep}	E_1		E_{pol} (RVS)	$E_{\mathrm{pol*}}$	E_{pol} (HF)	E_{pol}	E_{ct}	BSSE	$E_{\mathrm{ct*}}$	E_{ct}	ΔE	
Cy^-/Imh	7.6	6.1	4.2	8.8	11.8	14.9	−3.2	−3.7	−3.1	−3.6	−0.6	−0.4	−0.2	0.0	8.1	11.3
Cy^-/Cy^-	80.8	80.2	1.3	2.0	82.1	82.2	−5.7	−5.9	−5.1	−5.2	0.1	−0.3	0.4	0.0	77.1	77.0
Cy^-/Zn(II)	−312.9	−310.1	52.4	48.8	−260.5	−261.3	−76.3	−76.2	−79.6	−79.6	−51.1	−1.3	−49.8	−51.2	−391.3	−392.1
Cy^-/Ethoh	−2.3	−2.0	11.3	9.8	9.0	7.8	−3.8	−4.5	−3.8	−4.8	−1.6	−0.5	−1.1	−1.9	3.6	1.1
Imh/Cy^-	12.9	11.9	1.9	3.4	14.8	15.3	−3.0	−2.9	−2.8	−2.8	−0.1	−0.4	0.3	0.0	11.9	12.5
Imh/Zn(II)	−83.2	−83.9	20.7	20.9	−62.5	−63.0	−61.6	−52.0	−63.2	−53.7	−16.5	−0.5	−16.0	−15.0	−142.1	−131.7
Imh/Ethoh	3.1	1.3	1.6	3.0	4.7	4.3	−0.4	−0.4	−0.5	−0.4	−0.2	−0.4	0.2	0.0	4.0	3.9
Cy^-/Zn(II)	−317.9	−308.4	53.9	49.7	−264.0	−258.7	−76.7	−79.6	−79.9	−83.1	−51.2	−1.5	−49.7	−51.0	−395.1	−392.8
Cy^-/Ethoh	9.8	7.9	2.1	2.2	11.9	10.1	−2.4	−2.3	−2.3	−2.2	−0.3	−0.4	0.1	0.0	9.3	7.9
Ethoh/Zn(II)	−61.5	−60.9	22.6	20.3	−38.9	−40.6	−48.0	−44.5	−49.2	−45.7	−10.6	−0.6	−10.0	−8.1	−98.7	−94.5
sum	−663.6	−657.9	172.0	168.9	−491.6	−489.0	−281.1	−272.0	−289.5	−281.1	−132.1	−6.3	−125.8	−127.3	−913.2	−897.4
complex *a* (without Phe93)	−661.6	−657.9	177.4	168.9	−484.2	−489.0	−113.2	−121.7	−85.5	−94.5	−48.5	−5.1	−43.4	−41.3	−618.2	−624.9
δE_{nadd}	2.0	0.0	5.4	0.0	7.4	0.0	167.9	150.3	204.0	186.6	83.6	1.2	82.4	86.0	295.0	272.5
Cy^-/Benz	1.0	1.1	0.0	0.0	1.0	1.1	−0.3	−0.5	−0.3	−0.5	0.0	0.0	0.0	0.0	0.7	0.6
Imh/Benz	0.5	0.3	0.4	0.4	0.9	0.7	−0.1	−0.1	−0.1	−0.1	−0.1	−0.2	0.1	0.0	0.8	0.6
Benz/Cy^-	0.9	0.1	2.4	3.6	3.3	3.7	−3.2	−3.7	−3.2	−3.7	−0.5	−0.3	−0.2	0.0	−0.4	−0.1
Benz/Zn(II)	−5.1	−5.5	0.0	0.0	−5.1	−5.5	59.6	−6.9	4.9	−6.9	−69.7	0.0	−69.7	0.0	−69.9	−12.3
Benz/Ethoh	−0.1	−0.5	0.4	0.6	0.3	0.1	0.0	0.0	0.0	0.0	−0.1	−0.1	0.0	0.0	0.2	0.0
sum	−666.4	−662.4	175.2	173.5	−491.2	−488.9	−225.2	−283.1	−288.1	−292.2	−202.5	−6.9	−195.6	−127.3	−981.9	−908.6
complex *b* (with Phe93)	−664.4	−662.4	180.2	173.5	−484.2	−488.9	−111.8	−120.1	−84.5	−93.2	−48.6	−5.6	−43.0	−41.3	−617.3	−623.4
δE_{nadd}	2.0	0.0	5.0	0.0	7.0	0.0	113.4	163.0	203.6	199.0	153.9	1.3	152.6	86.0	364.6	285.2

[a] The IMP2 and MP2 energy gain, δE(IMP2) and δE(MP2), respectively, are also reported. The corresponding SIBFA values are recast for ease of comparison.

Its superimposition with the X-ray structure is represented in Figure 1b.

Results and Discussion

Table 1 reports the intermolecular interaction energies and their contributions in two Zn-tetracoordinated complexes, without and with, respectively, the involvement of the Phe93 side chain. These latter are denoted as complexes *a* and *b*, respectively. These energies were obtained at both QC/CEP 4−31G(2d) and SIBFA levels. Table 2 gives the values of the RVS intermolecular interaction energies in all bimolecular complexes as well as their individual contributions. The corresponding SIBFA interaction energies (without E_{disp}) are given in comparison. The values of nonadditivities, δE_{nadd}, are given as the difference between the summed bimolecular interaction energies and the value in the polycoordinated complex *a* or *b*.

$$\delta E_{\mathrm{nadd}} = \Delta E_{\mathrm{poly(many\text{-}body)}} - \sum \Delta E_{\mathrm{bimol(2\text{-}body)}} \qquad (2)$$

Table 3. Values (kcal/mol) of the CEP 4-31G(2d) RVS, MP2, and DFT Bimolecular Interaction Energies, of Their Sums, and Values of Their Nonadditivities[a]

	RVS	SIBFA	MP2	SIBFA	MP2	MP2	DFT	SIBFA
	ΔE		δE(MP2)	E_{disp}	ΔE (RVS) + δE(MP2)	ΔE	ΔE	ΔE_{tot}
Cy^-/Imh	8.1	11.3	−5.9	−3.0	2.3	1.8	5.3	8.3
Cy^-/Cy^-	77.1	77.0	−2.7	−3.2	74.3	73.9	75.5	73.8
Cy^-/Zn(II)	−391.3	−392.1	−20.6	−18.0	−411.9	−413.3	−445.5	−410.1
Cy^-/Ethoh	3.6	1.1	−5.8	−4.2	−2.2	−2.7	−0.4	−3.1
Imh/Cy^-	11.9	12.5	−3.8	−2.0	8.1	6.3	10.0	10.5
Imh/Zn(II)	−142.1	−131.7	−12.2	−7.3	−154.3	−154.8	−175.9	−139.0
Imh/Ethoh	4.0	3.9	−3.7	−1.6	0.4	−1.4	2.9	2.3
Cy^-/Zn(II)	−395.1	−392.8	−20.4	−18.3	−415.5	−417.1	−449.6	−411.1
Cy^-/Ethoh	9.3	7.9	−3.2	−1.8	6.1	5.6	7.3	6.1
Ethoh/Zn(II)	−98.7	−94.5	−8.8	−6.5	−107.5	−108.0	−126.7	−101.0
sum	−913.3	−897.4	−87.1	−65.8	−1000.4	−1009.7	−1097.2	−963.3
complex *a* (without Phe93)	−618.2	−624.9	−48.4	−65.8	−666.6	−673.1	−676.4	−690.8
δE_{nadd}	295.1	272.5	38.7	0.0	333.8	336.6	420.8	272.5
Cy^-/Ben	0.7	0.6	−0.8	−0.1	−0.1	−0.1	0.6	0.5
Imh/Ben	0.8	0.6	−3.6	−0.8	−2.8	−3.0	0.6	−0.2
Ben/Cy^-	−0.4	−0.1	−5.8	−2.7	−6.1	−7.7	−2.5	−2.7
Ben/Zn(II)	−69.9	−12.3	−47.6	−0.2	−117.5	−117.6	−140.3	−12.5
Ben/Ethoh	0.2	0.0	−2.5	−0.8	−2.3	−3.5	0.6	−0.7
sum	−981.9	−908.6	−147.3	−70.5	−1129.3	−1141.5	−1238.2	−978.9
complex *b* (with Phe93)	−617.3	−623.4	−57.8	−70.5	−675.1	−683.2	−676.7	−693.9
δE_{nadd}	364.6	285.2	89.5	0.0	454.2	458.3	561.5	285.0

[a] The MP2 energy gain, δE(MP2), is also reported. The corresponding SIBFA values are given along with their QC counterparts.

Positive δE_{nadd} values indicate anticooperativity. Table 3 regroups the intermolecular QC interaction energies at the HF level as well as at correlated levels, together with their SIBFA counterparts. Thus the CEP 4−31G(2d) are recast at HF and MP2 levels and complemented with the DFT results. The 6−311G** calculations are given at the HF, DFT, and MP2 levels, while the LACV3P** results are given at the HF, DFT, and lMP2 levels. Since we wish to compare trends, all QC computations were single-point computations done at the SIBFA-energy-minimized geometries.

In Table 1 two values of E_{pol} are given. E_{pol}(RVS) is the value of the summed monomer polarization energies at the RVS level and, as in ref 26, is compared to E_{pol*}(SIBFA), obtained prior to the iterative inclusion of the effects of the induced dipoles on the field. The Kitaura-Morokuma[27](denoted as KM) procedure strongly overestimates the polarization energy in the presence of strong electric fields such as those generated by metal cations due to a lack of fulfillment by the Pauli principle [see refs 11, 26c, and 28 and references therein]. Indeed, in that case, the repulsive exchange-polarization term is neglected as the wave function is not fully antisymmetrized. Therefore we have indirectly derived a value for E_{pol}(HF) after completion of the SCF cycles. Thus, from the converged interaction energy ΔE(HF), we subtracted the summed values of E_1 and E_{ct}. For such an evaluation, both ΔE(HF) and E_{ct} are uncorrected for BSSE effects[13] for consistency. E_{pol}(HF) is then compared to E_{pol}(SIBFA), derived at the end of the iterative process on the induced dipoles (see Table 1 for details). The comparisons between E_{pol}(HF) and E_{pol}(RVS), on the one hand, and between E_{pol}(SIBFA) and E_{pol*}(SIBFA), on the other hand, give insight into the contribution of induced dipoles to anticooperativity. It is seen that such a contribution has closely similar values from both QC and SIBFA calculations, namely in the 27.3−27.9 kcal/mol range. E_{ct*} denotes the value of E_{ct} after the BSSE correction. ΔE(RVS) and ΔE(SIBFA) denote the total QC and PMM intermolecular interaction energies prior to, respectively, the MP2 procedure and without the E_{disp} contribution. ΔE(MP2) and ΔE_{tot}(SIBFA) denote respectively the corresponding values after the MP2 procedure and with the E_{disp} contribution.

RVS Results. Table 1 shows that for both *a* and *b* complexes, a very close agreement between RVS and SIBFA obtains, consistent with previous studies.[14,22,26a,b] It bears on both the total energies and their individual contributions. The magnitude of ΔE(SIBFA) is larger than that of ΔE(RVS) by less than 1.5%. The trends in energy contributions upon including benzene are similar in the RVS and SIBFA approaches. In the context of each methodology, $E_{Coul.}/E_{MTP*}$ and E_{exch}/E_{rep} increase in magnitude by similar amounts. E_1 is seen to undergo a virtually null change with both approaches. E_{pol} decreases in magnitude by less than 1.5 kcal/mol out of 100, while E_{ct} is lowered by a negligible amount (< 0.4 kcal/mol). Thus the values of both ΔE(RVS) and ΔE(SIBFA) are modestly (<1.5 kcal/mol out of 620) decreased in magnitude by the involvement of the benzene ring. This could imply that, prior to including electron correlation/dispersion effects, indirect cation-π interactions involving benzene would weakly destabilize the complex rather than stabilize it. However, analysis of nonadditivity as reported below (Table 2) shows the present results to be only due to the anticooperativities of E_{pol} and E_{ct}: these result from the neutralization of the fields exerted on benzene by Zn(II), on the one hand, and by the two cysteinates, on the other hand.

MP2 Results. In complex *a*, δE(MP2) is smaller in magnitude than E_{disp}, namely −48.4 kcal/mol as compared to −65.9, owing to nonadditivity at the MP2 level (see below). This results in ΔE_{tot}(SIBFA) now being larger in magnitude than ΔE(MP2) by 3.5% instead of 1.5% at the RVS level. As shown below, larger relative energy differences can actually be found between the QC ΔE values depending upon the basis sets and the handling of correlation. This could be a concern owing to the large magnitudes of the absolute binding energies. In this connection, we have recently investigated the complexes formed between competing inhibitors and protein targets, such as Zn-metalloenzymes β-lactamase[29a] and phosphomannoisomerase.[29b] In model complexes extracted from the inhibitor-protein complexes, we found that, as in the present study, the SIBFA ΔE values differed from the CEP 4−31G(2d) target values by relative amounts of 2−3%, and slightly larger relative errors were observed between LACVP3** and CEP 4−31G(2d) ΔE(QC) values. Nevertheless, upon comparing the relative stabilities of several competing complexes for a given model site, the ΔE(QC) values from the two basis sets displayed parallel evolutions, and the ΔE(SIBFA) values very closely reproduced their trends and the energy ranking of the competing complexes. While these studies should be extended to other molecular recognition problems, such results indicate that a correct reproduction of relative energy differences and trends could be expectable from PMM. The corresponding values in complex *b* are −57.8 and −70.5 kcal/mol. Upon comparing the values of ΔE(MP2) and of ΔE_{tot}(SIBFA) in complexes *a* and *b*, it is seen that the benzene ring contributes −8.5 and −3.1 kcal/mol by MP2 and SIBFA computations, respectively. In the superoxide dismutase (SOD) binding site, a Trp residue interacts with the Fe(III) cofactor through a water molecule.[5] A QC study showed it to contribute by a larger amount (10 kcal/mol) to the stabilization energy[2] than computed here for ADH. However, a different balance of effects could come into play in the SOD site, since in contrast to ADH, the field exerted on the ring by a trivalent metal cation could now be incompletely neutralized by the anionic charges of the iron-coordinating Asp residue and azide molecule. The values of ΔE are very large since the present calculations are in the gas phase. Extrapolation to the actual ethanol-ADH complex would require the inclusion of the entire protein and perform energy balances taking into account the solvation energy of the complex, on the one hand, and the separate desolvation energies of the protein and the substrate prior to complex formation, on the other hand. Inclusion of the latter terms results in a considerable reduction of the magnitudes of the resulting binding energies. Accounting for the protein and ligand conformational energy rearrangement further reduces their magnitudes. Such energy balances have been reported concerning the complexation of inhibitors to the Zn-metalloproteins phosphomannoisomerase[29b] and the second Zn-finger of the HIV-1 nucleocapsid.[30] They resulted in binding energies in the range of −20 kcal/mol. Inclusion of entropy effects should further reduce their magnitudes. Nevertheless the trends in ΔE contributions regarding the effect of Phe93 should be conserved in the model site compared to the entire protein.

Analysis of Nonadditivity

Complex *a*. *1) QC Results.* Nonadditivity in several polycoordinated Zn(II) complexes was previously analyzed in parallel by RVS and SIBFA.[26a,b] Consistent with these studies, as shown in Table 2 E_{pol} is found here to be the most anticooperative contribution, with δE_{nadd} amounting to 168 and 204 kcal/mol for E_{pol}(RVS) and E_{pol}(KM), respectively. E_{ct} is also strongly anticooperative, with δE_{nadd} in the 83−87 kcal/mol range, while $E_{Coul.}$ and E_{exch} have very modest anticooperativities (2 and 5 kcal/mol, respectively). A small anticooperativity of the BSSE correction (1.3 kcal/mol out of 6) can be noted. Table 3 shows that the energy gain due to the MP2 procedure, δE(MP2), has a significant anticooperativity. It amounts to 38.7 kcal/mol and is in the same range as found in related polycoordinated Zn(II) complexes.[26a,b] Since such a value comes on top of the nonadditivities of E_{pol} and E_{ct} at the RVS level, it should stem mainly from the increases of the relative weights of both E_{pol} and E_{ct} due to correlation.[11] Further SIBFA studies are planned using correlated multipoles and polarizabilities [see for an example ref 21d]. They should allow quantification of the extent to which correlation affects the anticooperativities of E_{pol} and E_{ct} in the context of molecular mechanics. For this purpose, a preliminary recalibration of E_{pol} and E_{ct} on such monoligated Zn(II) complexes will be necessary and is outside the scope of this work. In a study of the complexes of nucleic acid base pairs with divalent metal cations, it was recalled[31] that the nonaddivity of the actual dispersion term appears only at the MP3 level[32] and is therefore not accounted for in the present calculations.

2) SIBFA Results. E_{pol} and E_{ct} are presently the only nonadditive SIBFA contributions. The values of δE_{nadd}(SIBFA) for E_{pol} and E_{ct} are consistent with the RVS ones. Those of E_{pol} and E_{pol*} are somewhat smaller than the corresponding RVS ones, but such underestimations are found to compensate for some corresponding underestimations of E_{pol}(SIBFA) with respect to E_{pol}(QC). This occurs notably in one binary complex, that of Zn(II) with imidazole at the 2.16 Å Zn−N distance. As commented on in ref 26, δE_{nadd} is larger when in the QM computations E_{pol} is derived at the outcome of the SCF procedure, and when in the SIBFA computations, it is computed after iterative inclusion of the induced dipoles. The value of δE_{nadd} for E_{ct} is very close to the corresponding RVS ones. This indicates that, compared to our previous calibration, the E_{ct} recalibration reported in ref 14 affords an improved control of its large nonadditivity in Zn(II) complexes.[26a,b]

Complex *b*. Quantifying nonadditivity by RVS/CEP 4−31G(2d) is prevented by the fact that at large separation, the bimolecular Zn(II)-benzene complex diverges asymptotically toward an open-shell state where an electron is transferred to the dication. In the present complex, the distance between Zn and the centroid of benzene is 5.4 Å. The RVS analysis gives a value of E_{ct} of −69.7 kcal/mol and an artifactual positive E_{pol} value of 59.6. Interposing the ethanol ligand, as in the trimolecular benzene-ethanol-Zn(II) complex, recovers a negative E_{pol} of −25.5 kcal/mol and a reduced E_{ct} value of −43.3 kcal/mol (unpublished). A fortiori, completion of the Zn(II) coordination shell recovers meaningful values of both contributions. The SIBFA computations show that the separate values of E_{pol} in the bimolecular complexes of benzene with Zn(II) and with one cysteinate are significant despite the distances of separation, amounting to −6.9 and −3.8 kcal/mol, respectively, but that the actual

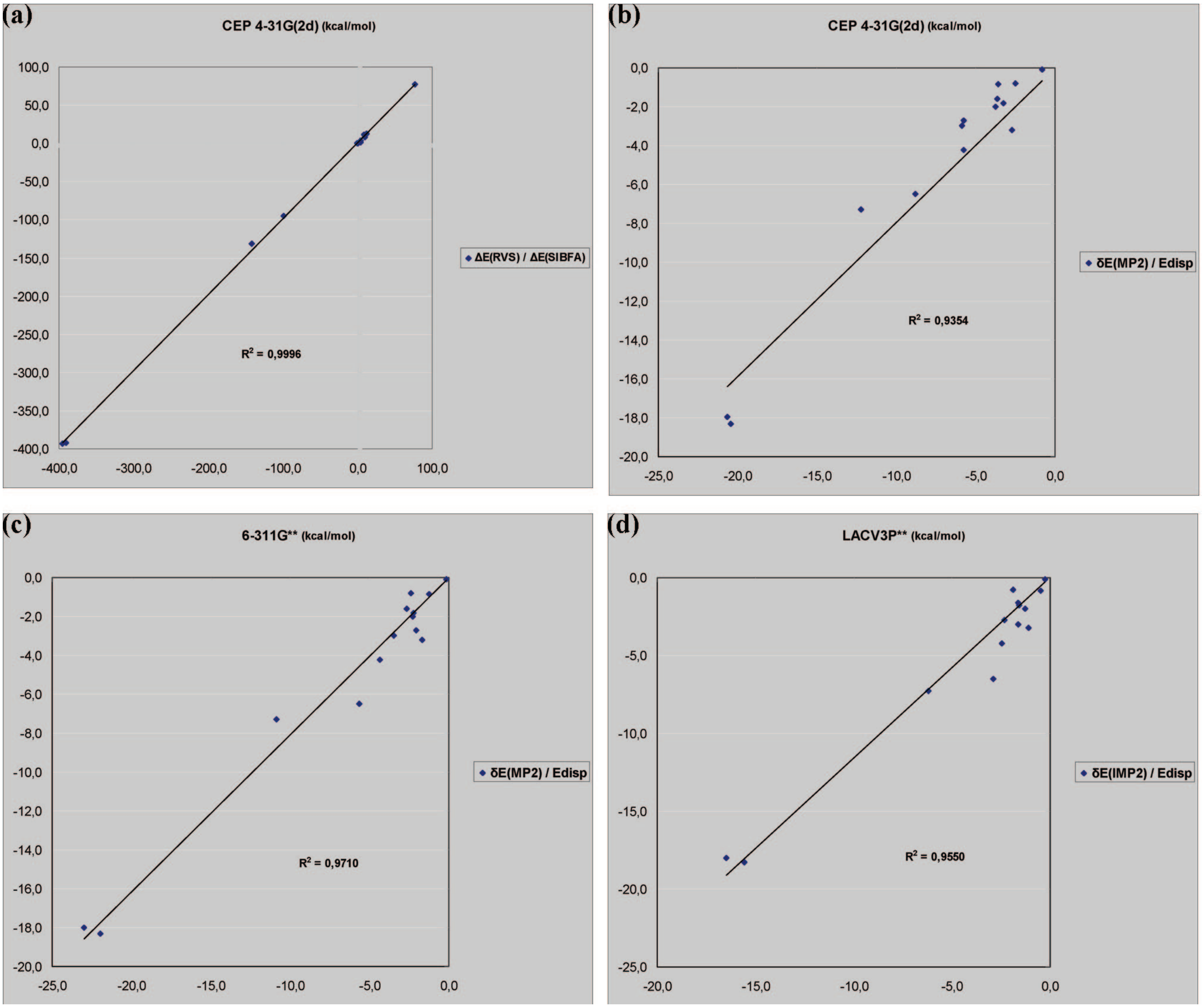

Figure 2. Correlation between the SIBFA and QC interaction energies in all bimolecular complexes except for Zn(II)-benzene. a) ΔE(SIBFA) and ΔE(RVS/HF); b) E_{disp}(SIBFA) and δE(MP2)/CEP4−31G(2d); c) E_{disp}(SIBFA) and δE(MP2)/6−311G**; and d) E_{disp}(SIBFA) and δE(lMP2)/LACV3P**.

increase of E_{pol} upon passing from complex *a* to *b* is negligible owing to the anticooperativity of E_{pol}. Thus in the context of SIBFA, this should leave E_{disp} as the sole energy contribution stabilizing complex *b* over complex *a*.

We have reported in Figure 2a the correlation between ΔE (RVS) and ΔE(SIBFA) bearing on all bimolecular complexes, except Zn(II)-benzene. The r^2 correlation coefficient is 0.9996. We have similarly evaluated the correlation between E_{disp}(SIBFA) and δE(MP2). E_{disp}(SIBFA) is an approximation to the real dispersion, since electron correlation affects also electrostatic and induction terms [see ref 11 and references therein]. Therefore a less satisfactory correlation has to be expected, especially as BSSE effects are in general not negligible. A reasonable r^2 of 0.9354 nevertheless is obtained with δE(MP2)/CEP 4−31G(2d) (Figure 2b), which actually increases to 0.97010 concerning δE(MP2)/6−311G** (Figure 2c). The r^2 value with respect to δE(lMP2)/LACV3P** is 0.9550 (Figure 2d). At this point it is recalled that the calibration of E_{disp}(SIBFA) was performed[33] on the basis of SAPT computations; however, SAPT can become intractable upon increasing the size of the molecular complexes. Alternatively, MPn ($n = 3$ or 4) or CCSD(T) computations could be used for E_{disp}(SIBFA) recalibration on model dimeric complexes. There could be two means to improve the representation of correlation in SIBFA. One is a simple rescaling on the basis of such more extended correlated computations. The second is, as mentioned above, the use of correlated multipoles and polarizabilities:[21d] these could provide the contributions of correlation to E_{MTP} and E_{pol}. A rescaling of E_{disp} should be done subsequently to provide the actual contribution of the van der Waals component.

Effects of the Level of the QC Computations (Tables 3 and 4). This analysis was performed in order to evaluate, on the one hand, the sensitivity of δE_{nadd} to the level of the QC computation, and, on the other hand, the amount of stabilization due to correlation in both complexes *a* and *b*. In complex *b*, the 'van der Waals' component should be amplified, which would lead to ΔE underestimation by DFT [see ref 34 and references therein]. On the other hand, complex *a* is predominantly stabilized by electrostatic interactions, so that all QC procedures could be expected to

Table 4. Values (kcal/mol) of the LACV3P** HF, IMP2, and DFT Bimolecular Interaction Energies, of Their Sums, and Values of Their Nonadditivities and Corresponding Values of the 6-311G** HF and MP2[a]

	LACV3P**							6−311G**					
	HF	SIBFA	IMP2	SIBFA	IMP2	DFT	SIBFA	HF	SIBFA	MP2	SIBFA	MP2	SIBFA
	ΔE		δE(IMP2)	E_{disp}	ΔE		ΔE_{tot}	ΔE		δE(MP2)	E_{disp}	ΔE	ΔE_{tot}
Cy$^-$/Imh	8.4	11.3	−1.6	−3.0	6.7	6.9	8.3	8.3	11.3	−3.5	−3.0	4.8	8.3
Cy$^-$/Cy$^-$	78.4	77.0	−1.1	−3.2	77.3	77.3	73.8	78.4	77.0	−1.7	−3.2	76.7	73.8
Cy$^-$/Zn(II)	−378.5	−392.1	−16.5	−18.0	−395.0	−407.0	−410.1	−368.4	−392.1	−23.0	−18.0	−391.4	−410.1
Cy$^-$/Ethoh	4.3	1.1	−2.5	−4.2	1.9	0.4	−3.1	4.3	1.1	−4.3	−4.2	0.0	−3.1
Imh/Cy$^-$	12.6	12.5	−1.3	−2.0	11.4	11.4	10.5	12.5	12.5	−2.3	−2.0	10.2	10.5
Imh/Zn(II)	−137.7	−131.7	−6.2	−7.3	−143.9	−155.9	−139.0	−133.7	−131.7	−10.9	−7.3	−144.6	−139.0
Imh/Ethoh	3.6	3.9	−1.6	−1.6	1.9	2.6	2.3	3.5	3.9	−2.6	−1.6	0.9	2.3
Cy$^-$/Zn(II)	−382.5	−392.8	−15.6	−18.3	−398.1	−410.9	−411.1	−372.3	−392.8	−21.9	−18.3	−394.2	−411.1
Cy$^-$/Ethoh	10.2	7.9	−1.6	−1.8	8.6	8.2	6.1	10.1	7.9	−2.2	−1.8	7.9	6.1
Ethoh/Zn(II)	−97.2	−94.5	−2.9	−6.5	−100.1	−112.2	−101.0	−93.5	−94.5	−5.7	−6.5	−99.2	−101.0
sum	−878.5	−896.7	−50.8	−65.8	−929.3	−979.3	−962.5	−850.9	−962.5	−78.1	−65.8	−929.0	−962.5
complex *a* (without Phe93)	−607.9	−624.9	−20.8	−65.8	−628.8	−629.5	−690.8	−594.7	−624.9	−35.4	−65.8	−630.1	−690.8
δE_{nadd}	270.6	272.0	30.0	0.0	300.5	349.9	271.9	256.2	271.9	42.7	0.0	298.9	271.9
Cy$^-$/Ben	0.8	0.6	−0.2	−0.1	0.5	0.6	0.5	0.8	0.6	−0.2	−0.1	0.6	0.5
Imh/Ben	0.5	0.6	−0.5	−0.8		0.4	−0.2	0.5	0.6	−1.3	−0.8	−0.7	−0.2
Ben/Cy$^-$	0.3	−1.5	−2.3	−2.7	−2.1	−0.9	−2.7	0.3	−1.5	−2.0	−2.7	−2.6	−2.7
Ben/Zn(II)	−63.5	−12.3	−30.7	−0.2	−94.1	−114.4	−12.5	−60.3	−12.3	−44.1	−0.2	−105.1	−12.5
Ben/Ethoh	0.0	0.7	−1.9	−0.8	−1.9	−0.1	−0.7	0.0	0.7	−2.4	−0.8	−1.1	−0.7
sum	−940.4	−908.5	−908.5	−70.5	−1026.9	−1093.7	−978.1	−909.5	−978.1	−128.0	−70.5	−1037.9	−978.1
complex *b* (with Phe93)	−608.0	−623.4	−24.1	−70.5	−632.1	−630.1	−693.9	−595.0	−623.4	−40.3	−70.5	−635.3	−693.9
δE_{nadd}	332.4	285.3	285.3	0.0	394.8	463.5	284.4	314.5	284.4	87.7	0.0	402.6	284.4

[a] The IMP2 and MP2 energy gains, δE(IMP2) and δE(MP2), respectively, are also reported. The corresponding SIBFA values are recast for ease of comparison.

show similar trends. The 6−311G** and LACV3P** computations differ by the use of an effective large core pseudopotential on Zn(II) in the latter, while a full electron basis set is used on the cation in the 6−311G** calculations. Table 3 reports the values of the total interaction energies and those of all bimolecular complexes at the CEP 4−31G(2d) level, and Table 4 reports the corresponding values at the LACV3P** and 6−311G** levels.

Complex *a*. At the HF level, the magnitudes of the interaction energies are along the sequence CEP 4−31G(2d) > LACV3P** > 6−311G**. The 10 out of −620 kcal/mol energy difference between the first two basis sets is the same as the corresponding one previously computed for the complex of Zn(II) with two cysteinates and two imidazoles that represented a Zn-finger Zn binding site.[14] The magnitudes of anticooperativity effects follow the same trend as the ΔE values. Such larger δE_{nadd} values with the CEP 4−31G(2d) basis set translate the larger relative weights of the summed second-order contributions with respect to the summed first-order ones, occurring with this basis set compared to the LACV3P and 6−311G** ones. It could be due to the presence in this basis of two diffuse 3d polarization AOs on the heavy atoms.

In Table 3 two columns of MP2 values are given, namely columns 5 and 6 of results. The first column gives the results after addition of the MP2 energy gain, δE(MP2), to ΔE(RVS), i.e., after BSSE correction at the HF level. The second column gives the corresponding results after the addition of δE(MP2) to ΔE(HF), namely, without the BSSE(HF) correction. Thus the energy values are slightly smaller than in the preceding column. The penultimate column gives the DFT results, and the last column recasts the SIBFA ones. At the MP2 level, the total interaction energies are larger with the CEP 4−31G(2d) than with the 6−311G** basis set, but the δE_{nadd} values are close, 38.7 and 42.7 kcal/mol, respectively. The larger magnitudes of ΔE(MP2) with the CEP 4−31G(2d) basis set stem from their larger magnitudes in the separate monoligated Zn(II) complexes. It is noted that for these complexes at optimized Zn-ligand distances the values of ΔE(MP2) using the CEP 4−31G(2d) set[35] are found to be very close to the large basis set computations recently published by Rayon et al. on a series of representative Zn-ligand complexes.[36] These computations used aug-cc-pVTZ basis sets with both MP2 and CCSD(T) methods.

At the DFT level, the CEP 4−31G(2d) basis set has a larger δE_{nadd} than the LACV3P** basis set (420.8 versus 349.9 kcal/mol), the 71 kcal/mol difference being amplified with respect to the corresponding HF δE_{nadd} value which amounted to 24.5 kcal/mol. With both CEP 4−31G(2d) and LACV3P** basis sets, the DFT computations are seen to overestimate the Zn-monoligand interaction energies. In this connection, recent analyses[11] of DFT intermolecular interaction energies with the Constrained Space Orbital Variation procedure[37] linked these overestimations to a strong increase of polarization, charge-transfer contributions, and Zn(II) polarizability as compared to the corresponding HF values. Overestimations of ΔE were recently also noted in the case of Zn(II) complexes with anionic ligands,[36] while new functionals are being developed and evaluated.[38] Using B3LYP and the CEP 4−31G(2d) basis set, the DFT larger δE_{nadd} values compared to MP2 compensate for the larger monoligated ΔE(DFT) values. As a result, the final ΔE(DFT) values come close to the ΔE(MP2) ones, −676.4 as compared to −666.6 kcal/mol.

At the lMP2 level, and with the LACV3P** basis set, ΔE(lMP2) has in the monoligated Zn(II) complexes smaller values than ΔE(DFT) but also a smaller δE_{nadd} value: this mutual compensation results in ΔE(lMP2) and ΔE(DFT) being virtually equal in the polycoordinated complex.

Complex *b*. We compare here the contribution of the benzene ring to stabilization, as translated by the energy variations upon passing from complex *a* to complex *b*. It will be denoted $\delta E_{\mathrm{a\text{-}b}}$. At the HF level, and consistent with the CEP 4−31G(2d) results, both 6−311G** and LACV3P** basis sets indicate the benzene ring to contribute negligibly to the interaction energy. This occurs in spite of the artifactually strong ΔE value in the 'bimolecular' Zn(II)-benzene complex of −60 to −63.5 kcal/mol, comparable to the corresponding CEP 4−31G(2d) value of −70 kcal/mol value.

At the MP2 level, $\delta E_{\mathrm{a\text{-}b}}$/6−311G** amounts to −5.2 kcal/mol. This value is smaller in magnitude than the $\delta E_{\mathrm{a\text{-}b}}$/CEP 4−31G(2d) value of −8.5 kcal/mol but closer to the SIBFA value of −3.1 kcal/mol. Concerning the bimolecular complexes involving benzene with ethanol, each cysteinate as well as imidazole, at the HF level, ΔE/CEP 4−31G(2d) is seen to be only slightly more stabilizing than ΔE computed with the larger 6−311G** basis set. However the corresponding energy differences are enhanced at the MP2 level, regardless of the relative proximity to benzene. This illustrates the need for extended basis sets in order to handle correlation. Concerning the complexes between two conjugated molecules, it was shown by Hobza and Sponer[39] that extrapolation to the complete basis set (CBS) limit is necessary to obtain converged estimates of the MP2 interaction energy as well as stable MP2-CCSD(T) energy differences. The CCSD(T) interaction energies have for such complexes smaller magnitudes than the MP2 ones. The fact that, for the complexes involving benzene, E_{disp}(SIBFA) in its present formulation is closer in magnitude to δE(MP2) computed with the 6−311G** than to the CEP 4−31G(2d) constitutes thus a favorable feature. Furthermore, as previously observed in a series of H-bonded complexes using the CEP 4−31G(2d) basis set,[15] the overestimations of ΔE(MP2)/CEP 4−31G(2d) can stem in part from large BSSE effects at the MP2 level, in marked contrast with the small CEP 4−31G(2d) BSSE magnitudes at the HF level. These caveats are to be noted, while, on the other hand and as abovementioned, the Zn(II) monoligated interaction energies can be accurately computed at the MP2/CEP 4−31G(2d) basis set.

$\delta E_{\mathrm{a\text{-}b}}$ at the DFT level has extremely small magnitudes (<0.3 kcal/mol) with both CEP 4−31G(2d) and LACV3P** basis sets. By contrast, at the lMP2 level, $\delta E_{\mathrm{a\text{-}b}}$ (LACV3P**) amounts to −3.3 kcal/mol.

Conclusions

We have analyzed by SIBFA and QC computations the energetical factors stabilizing the Zn-binding site of alcohol dehydrogenase (ADH), in which Zn(II) is polycoordinated to two cysteinates and one histidine and by the ethanol substrate. A Phe residue is stacked over ethanol. The stabilization energy it contributes was computed to be in the range of 3−9 kcal/mol. However, because of the mutual cancelation of the fields polarizing the benzene ring in the ADH binding site as compared to the separate bimolecular complexes involving it, no stabilization was computed in the context of QC/HF calculations and, concerning the SIBFA procedure, in the absence of the E_{disp} contribution.

Regarding nonadditivity, the present analyses have shown, in unanticipated fashion, some significantly differing behaviors of QC depending upon the level of computations. Thus DFT was found to display much larger anticooperativity than either MP2 in CEP 4−31G(2d) computations or lMP2 in LACV3P** computations. However such larger δE_{nadd} values were in both cases found to compensate for the larger DFT magnitudes of the separate Zn-monoligated complexes: with the CEP 4−31G(2d) basis set, this resulted in ΔE(DFT) differing from ΔE(MP2) by small amounts, namely 9.8 kcal/mol out of 670 in complex *a* and 1.6 kcal/mol out of 675 in complex *b*. On the other hand, more conservatively, δE_{nadd}(MP2) was found to have very similar values with either CEP 4−31G(2d) and 6−311G** basis sets, namely 38.7 and 42.7 kcal/mol, respectively, in *a* and 89.5 and 87.7 kcal/mol in *b*. The lMP2 computations with the LACV3P** basis set had smaller corresponding δE_{nadd} values of 29.8 and 62.4 kcal/mol. The large δE_{nadd} values in complex *b* are due to the artificially strong E_{ct} value in the benzene-Zn(II) complex which are not in direct interaction and the onset of an open-shell state where an electron is transferred to Zn(II).

The present investigation also confirms the accuracy of the SIBFA procedure into reproducing its target QC/CEP 4−31G(2d) interaction energies and a good control of both E_{pol} and E_{ct} nonadditivities. The relative error is 1.5% concerning the HF level. It raises however to 3.5% at the correlated level, because E_{disp} is additive, while δE(MP2) in polycoordinated Zn(II) complexes is anticooperative.[26a,b] The −3.1 kcal/mol stabilization contributed by the benzene ring appears closer to the 6−311G** than the CEP 4−31G(2d) MP2 value (−5.2 and −8.5 kcal/mol, respectively). In light of the results published by Hobza and Sponer,[39] it is likely that the 6−311G** stabilization energy of −5.2 kcal/mol is closer to the CBS result than the CEP 4−31G(2d) one and that its magnitude is itself an upper bound to the CCSD(T) value.

Polarization is indispensable to reliably compute cation-π complexes where an aromatic ring directly interacts with a cationic partner.[40] To our knowledge, the very first evaluation of E_{pol} in such complexes was published in 1980 upon studying the complexes of mono- and tetramethylammonium with the indole ring.[41] However the present study shows that, as concerns the ADH binding site, the contribution of the benzene ring to overall stabilization is not due to polarization. This contribution is canceled out because the dicationic charge, with which benzene interacts indirectly, is neutralized by the two anionic cysteinates. The main contribution then stems from dispersion, as in classical, nonpolarizable force-fields. Thus the present analysis reaffirms the need for a complete separability of the interaction potential[21d,22] in order to accurately reproduce each of the QC contributions to the binding energy in a diversity of situations (see Table 1).

Finally, the present results also suggest that PMM procedures which can accurately reproduce the results from QC computations could be used as a tool to refine X-ray crystal structures, as was previously demonstrated in the context of quantum chemistry[42] or by the use of distributed multipoles.[43,44] In view of such an evaluation, and as we had done in previous papers,[29] we give as Supporting Information the coordinates of the SIBFA energy-minimized structure. These could also be used to benchmark other polarizable molecular mechanics approaches.

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Supporting Information Available: Cartesian coordinates of the energy-minimized structure of complex *b*. This material is available free of charge via the Internet at http://pubs.acs.org.

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